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Comparative Study of Antioxidant Activity of Turmeric Ethanol Extract (*Curcuma longa* L.) and Ethanol Extract of Curcuma (*Curcuma xanthorrhiza* Roxb.) Using the *1.1-Diphenyl-2-Picrylhydrazil* (DPPH) Radical Scavenging Method

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Abstract. Antioxidants can scavenge free radicals, cancer prevention, and premature aging. One of the uses of antioxidant compounds can be found in the Zingiberaceae family, for example, turmeric (Curcuma longa L.) and Curcuma (Curcuma xanthorrhiza Roxb.). The sample used in this research is turmeric (*Curcuma longa* L.) and Curcuma (*Curcuma xanthorrhiza* Roxb.) which is included in the Zingiberaceae family. This study aimed to compare the antioxidant activity of turmeric ethanol extract (*Curcuma longa* L.) and Curcuma (*Curcuma xanthorrhiza* Roxb.) by using the radical 1,*1-Diphenyl-2-Picrylhydrazil* Scavenging Method. Extraction of turmeric and Curcuma using maceration method with 96% ethanol solvent and obtained yield value of 15.75% of turmeric ethanol extract and 13.44% of Curcuma ethanol extract. Qualitative analysis was performed by thin-layer chromatography method which was characterized by yellow stain after being sprayed of *1,1-Diphenyl*-2-*Picrylhydrazil* solution. Quantitative antioxidant activity test using UV-Vis spectrophotometric method measured at 515 nm wavelength. The results showed that turmeric ethanol extract and Curcuma ethanol extract had different antioxidant activities, where turmeric had a weak antioxidant activity with IC_{50} value 155,78µg/mL and Curcuma had strong antioxidant activity with IC_{50} value 26.38µg/mL

INTRODUCTION

Based on the source, antioxidants are divided into endogenous antioxidants, namely enzymes with antioxidant properties, such as Superoxide Dismutase (SOD), catalase (Cat), and glutathione peroxidase (Gpx) [1]; as well as exogenous antioxidants, which are obtained from outside the body/food. Various natural ingredients native to Indonesia contain many antioxidants with various active ingredients, including vitamins C, E, pro-vitamin A, organosulfur, -tocopherol, flavonoids, thymoquinone, statins, niacin, phycocyanin, and others [2]. Various natural ingredients, both those that have long been used as daily food or have just been developed as dietary supplements, contain these various antioxidants [3].

Antioxidants are needed to prevent oxidative stress. Oxidative stress is a condition of an imbalance between the amount of free radicals present and the number of antioxidants in the body [4]. Free radicals are compounds that contain one or more unpaired electrons in their orbitals, so they are highly reactive and capable of oxidizing surrounding molecules (lipids, proteins, DNA, and carbohydrates) [5]. Antioxidants are very easily oxidized, so free radicals will oxidize antioxidants and protect other molecules in cells from damage caused by oxidation by free radicals or reactive oxygen [6].

Antioxidants can scavenge free radicals, cancer prevention, and premature aging [7]. One of the uses of antioxidant compounds can be found in the Zingiberaceae family, for example, turmeric (*Curcuma longa* L.) and Curcuma (*Curcuma xanthorrhiza* Roxb.). Part of turmeric (*Curcuma longa* L.) and curcuma (*Curcuma xanthorrhiza* Roxb.) Which are mainly used is the rhizome which is used for the purposes of traditional medicinal herbs, among others for

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itching, atrophy, swollen gums, wounds, shortness of breath, stomach ache, ulcers, scabies, jaundice, improves digestion, antidiarrheal and antidote [8].

Secondary metabolites that play a role in various pharmacological effects caused by turmeric (*Curcuma longa* L.) and Curcuma (*Curcuma xanthorrhiza* Roxb.) are curcuminoids [4]. Curcuminoids are a group of diarylheptanoid compounds that are responsible for causing a yellow to orange color in the rhizomes of turmeric (*Curcuma longa* L.) and Curcuma (*Curcuma xanthorrhiza* Roxb.) [9]. The composition of compounds can be different in the same species depending on plant age, cultivation method, environmental conditions in which it is grown (geographic origin), harvest time, and post-harvest and storage processes [10].

METHODS

In this study, laboratory experiment measurement and analysis methods were used to obtain precise results. The sample used in this research is turmeric (Curcuma longa L.) and curcuma (Curcuma xanthorrhiza Roxb.) which are included in the Zingiberaceae family. This study aimed to compare the antioxidant activity of turmeric ethanol extract (Curcuma longa L.) and curcuma (Curcuma xanthorrhiza Roxb.) by using the radical 1,1-Diphenyl-2-Picrylhydrazil Scavenging Method. The steps or procedures in this study can be seen in Figure 1.

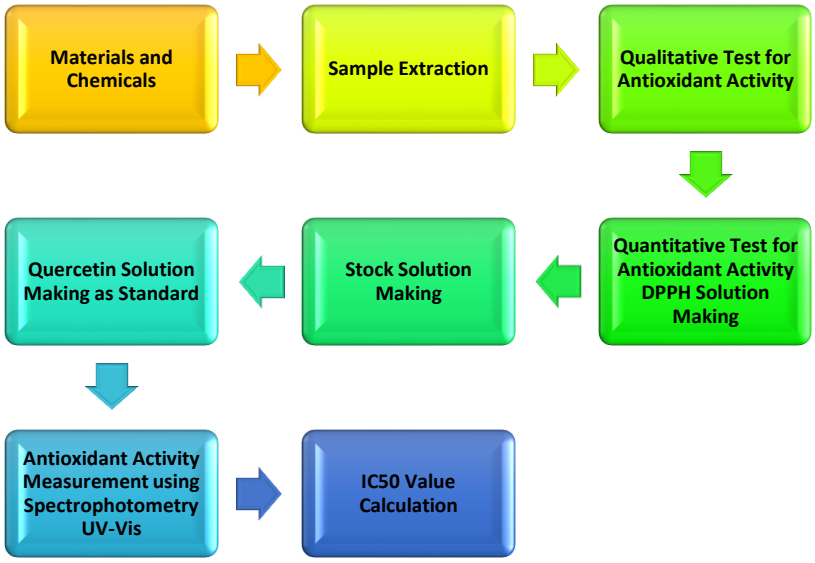

FIGURE 1. The procedures of laboratory experiment measurement and analysis methods in this study

Materials and Chemicals

Sample of turmeric (*Curcuma longa* L.) and Curcuma (*Curcuma xanthorrhiza* Roxb.) which have been obtained from Maros Regency,South Sulawesi Province.

Sample Extraction

Each sample of turmeric (*Curcuma longa* L.) and Curcuma (*Curcuma xanthorrhiza* Roxb.) were washed using running water, then thinly sliced with a thickness of ± 5 mm. After that the sample was dried in an oven until dry with temperature of 40°C. After dried, the sample is made into a powder. Sample extraction was carried out using maceration for 3x24 hours, stirred occasionally [2]. The ratio of sample weight to 96% ethanol solvent is 1:10. The liquid extract was concentrated using a rotary vacuum evaporator [9].

Qualitative Test for Antioxidant Activity

The ethanol extract of turmeric (*Curcuma longa* L.) and the ethanol extract of Curcuma (*Curcuma xanthorrhiza* Roxb.) were each dotted on the TLC plate of silica gel 60 F254, 7 x 1 cm using a capillary tube, then eluted with n-hexane: ethyl acetate (7: 3). After that the plates were tested for their antioxidant activity by sprayed with DPPH solution and allowed to stand for 30 minutes. Stains that have antioxidant activity will change from purple to yellow.

Quantitative Test for Antioxidant Activity DPPH Solution Making

3 mg of DPPH powder dissolved with 100 mL of methanol p.a in a measuring flask to obtain a DPPH solution with a concentration of 30 ppm.

Stock Solution Making

A stock solution of 1000 ppm was made by weighing the ethanol extract of turmeric (*Curcuma longa* L.) and ethanol extract of Curcuma (*Curcuma xanthorrhiza* Roxb.) 10 mg each and dissolved with methanol p.a while stirring and homogenizing then add the volume up to 10 mL

Quercetin Solution Making as Standard

10 mg of quercetin powder was dissolved with 10 mL of methanol p.a in a flask while stirred and homogenized, the volume was added to the limit mark to obtain a stock solution with a concentration of 1000 ppm. Then dilution was carried out with a series of concentrations of 2, 4, 6, 8, and 10 ppm by pipetting 0.01 mL, 0.02 mL, 0.03 mL, 0.04 mL, and 0.05 mL respectively. The concentration series and the volume is added to 5 mL. The concentration series that had been made were each pipetted 0.5 mL then added 3.5 mL of 30 ppm DPPH solution. The mixed solution was incubated for 30 minutes in a dark room then the absorption was measured at a wavelength of 515 nm.

Antioxidant Activity Measurement Using Spectrophotometry Uv-Vis

The stock solution that has been made is then diluted with a concentration series of 20, 40, 60, 80, and 100 ppm. For a concentration of 20 ppm pipetted 0.1 mL, concentration 40 ppm pipetted 0.2 mL, concentration 60 ppm pipetted 0.3 mL, concentration 80 ppm pipetted 0.4 mL, and concentration 100 ppm pipetted 0.5 mL, then add with methanol pa up to 5 mL. The concentration series that had been made were each pipette 0.5 mL then added 3.5 mL of 30 ppm DPPH. The solution was incubated in a dark room for 30 minutes, measured its absorbance at a wavelength of 515 nm.

IC_{50} Value Calculation

The percentage of DPPH radical inhibition is calculated by the formula:

$$\text{Inhibition } \% = \frac{[A_o - (A_1 - A_s)]}{A_o} \text{ x } 100\%$$

Where Ao is the absorbance of the blank, A1 is the absorbance containing the sample and DPPH, A2 is the absorbance of the sample without DPPH [10].

The IC50 value is a number that shows the concentration of the test sample which provides a reduction of 50% (able to inhibit or reduce the oxidation process by 50%). The IC50 value is determined by making a linear curve between the concentration of the test solution (x-axis) and the% of damping (y-axis) of the equation y = a + bx, the IC50 value can be calculated using the formula:

$$IC_{50} = \frac{(50\text{-}a)}{b}$$

RESULT AND DISCUSSION

In this study, the initial stage carried out to test the antioxidant activity of the ethanol extract of turmeric (*Curcuma longa* L.) and the ethanol extract of Curcuma (*Curcuma xanthorrhiza* Roxb.) is plant determination which aims to ensure that the plant sample used is indeed turmeric (Curcuma longa.) L.) and Curcuma (*Curcuma xanthorrhiza* Roxb.). The results of the determination obtained by the plants used in this study were the species *Curcuma longa* L. and the species *Curcuma xanthorrhiza* Roxb [11].

The next step is making turmeric extract (*Curcuma longa* L.) and Curcuma extract (*Curcuma xanthorrhiza* Roxb.) By maceration method using 96% ethanol solvent. The results obtained from the extraction of turmeric (*Curcuma longa* L.) and Curcuma (*Curcuma xanthorrhiza* Roxb.) can be seen in the table below:

TABLE 1. Yield value of the ethanol extract of turmeric (*Curcuma longa* L.) and the ethanol extract of Curcuma (*Curcuma xanthorrhiza* Roxb.)

Sample	Sample weight (g)	Amount of solvent (ml)	Extract weight (g)	Yield value (%)
Turmeric (*Curcuma longa* L.)	300	3000	47,278	15,759
Curcuma(*Curcuma xanthorrhiza* Roxb.)	300	3000	40,347	13,449

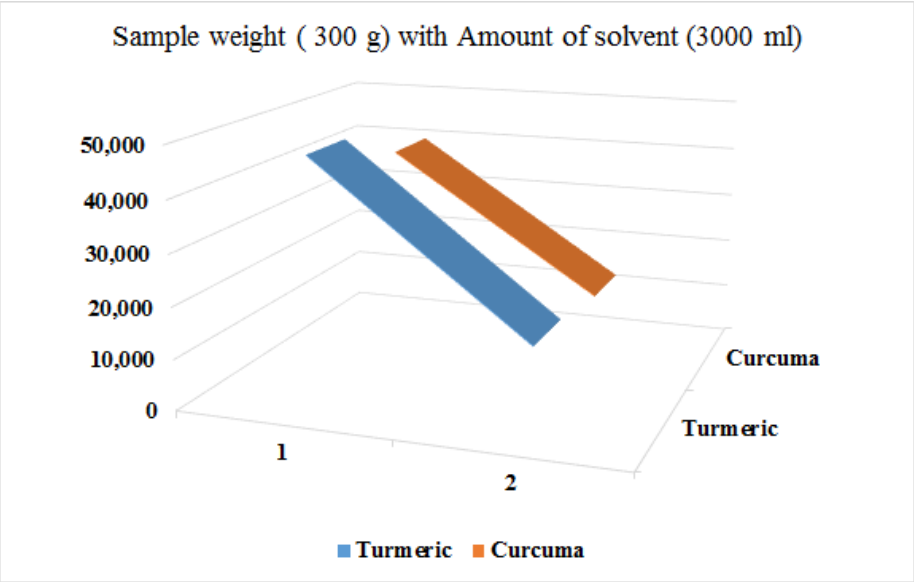

FIGURE 2. The relationship between the yield value of turmeric (*Curcuma longa* L.) and Curcuma (*Curcuma xanthorrhiza* Roxb.)

Turmeric (*Curcuma longa* L.) and Curcuma (*Curcuma xanthorrhiza* Roxb.) are plants that have antioxidant activity, this is because the rhizomes contain antioxidant compounds such as curcuminoids [12]. This study aims to compare the level of antioxidant activity between the ethanol extract of turmeric (*Curcuma longa* L.) and the ethanol extract of Curcuma (*Curcuma xanthorrhiza* Roxb.) from Maros Regency, South Sulawesi Province.

Qualitative tests were conducted to determine the presence of antioxidant compounds contained in the ethanol extract of turmeric (*Curcuma longa* L.) and ethanol extract of Curcuma (*Curcuma xanthorrhiza* Roxb.) Which was carried out using the thin layer chromatography (*TLC*) method. Thin layer chromatography is one of the analytical methods used to rapidly separate components based on the principles of adsorption and partition [2]. The qualitative

test results of the antioxidant activity of the ethanol extract of turmeric (*Curcuma longa* L.) and the ethanol extract of Curcuma (*Curcuma xanthorrhiza* Roxb.) can be seen in Table 2.

TABLE 2. Qualitative test results of the antioxidant activity of the ethanol extract of turmeric (*Curcuma longa* L.) and the ethanol extract of Curcuma (*Curcuma xanthorrhiza* Roxb.)

Sample	Spraying solvent	Result
Turmeric (*Curcuma longa* L.)	DPPH	(+) yellow stain, purple background
Curcuma(*Curcuma xanthorrhiza* Roxb.)	DPPH	(+) yellow stain, purple background

Note:
(+) : Positive antioxidant
(-) : Negative antioxidant

Stains that have antioxidant activity will change from purple to yellow. As according to [11], the ability of radical scavenging is related to the ability of the compound components to donate electrons or hydrogen. Any molecule that can donate electrons or hydrogen will react and will fade the DPPH color, where the DPPH intensity will change from purple to yellow by electrons coming from antioxidant compounds. Based on the results of research conducted qualitatively, the ethanol extract of turmeric (*Curcuma longa* L.) and the ethanol extract of Curcuma (*Curcuma xanthorrhiza* Roxb.) have antioxidant activity.

Determination of antioxidant activity was carried out in this study, it was found that the antioxidant compounds in temulawak extract such as curcuminoids, α-curcumene, ar-turmerone, and xanthorrhizol act as antioxidants that convert radicals into stable forms through hydrogen radical transfer. The initial stage to test antioxidant activity is the measurement of the maximum wavelength (λ maks). The maximum wavelength of the DPPH used is 515 nm. According to [12] the maximum wavelength of DPPH can be used because the maximum wavelength that can be used for measurements with the DPPH method is from 515 nm to 520 nm. The aim of this is to do incubation for 30 minutes so that the reaction between the sample solution and the DPPH solution takes place perfectly before measuring its antioxidant activity using a UV-Vis spectrophotometer. The results of measurements of absorbance, percent inhibition and IC50 values can be seen in Table 3.

TABLE 3. The results of measurements of absorbance, percent inhibition and IC50 values

Sample	Concentration (ppm)	(A) Sample and DPPH	(A) Sample	Inhibition % (%)	IC_{50} (µg/mL)
Turmeric (*Curcuma longa* L.)	20	0,615	0,005	26,594	155,780
	40	0,596	0,016	30,204	
	60	0,577	0,022	33,213	
	80	0,555	0,027	36,462	
	100	0,523	0,031	40,794	
Curcuma(*Curcuma xanthorrhiza* Roxb.)	20	0,630	0,152	42,478	26,386
	40	0,605	0,320	65,703	
	60	0,581	0,479	87,725	
	80	0,563	0,632	108,303	
	100	0,540	0,805	131,889	
Quercetin	2	0,636	0,002	23,706	14,933
	4	0,601	0,003	28,038	
	6	0,574	0,004	31,407	
	8	0,542	0,005	35,379	
	10	0,501	0,006	40,433	

Note:
Blank absorbance : 0,831

According to [9], a compound is declared as a very strong antioxidant if the IC50 value is <10 µg / mL, strong if the IC50 value is between 10-50 µg / mL, moderate if the IC50 value ranges from 50-100 µg / mL, it is weak if the IC50 value ranged from 100-250 µg / mL and was inactive if the IC50 was above 250 µg / mL.

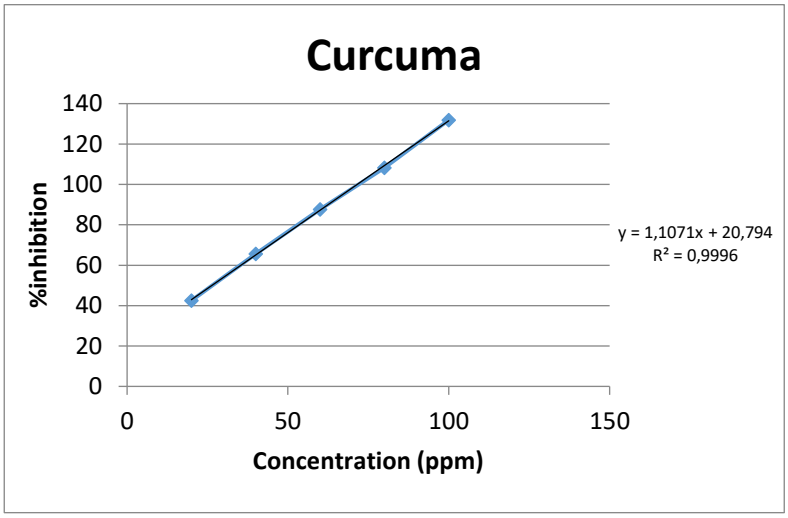

FIGURE 3. Relationship between the concentration of turmeric (*Curcuma longa* L.) ethanol extract and the percentage of DPPH inhibition

The sample concentrations used were 20, 40, 60, 80, and 100 ppm (Figure 3-5). This concentration was chosen in order to know what concentration the sample could inhibit 50% of the DPPH radical or so-called the IC50 value, where the purpose of calculating the IC50 value was to determine the level of antioxidant activity of a sample [13]. While the comparison used in this study was quercetin with a concentration of 2, 4, 6, 8 and 10 ppm, where this comparison was an aglycone compound from routine molecules without glycosides and the largest flavonol compound found in almost every type of plant [4].

The results obtained were based on measurements with a UV-Vis spectrophotometer at a wavelength of 515 nm, namely the IC50 quercetin value obtained was 14.933 µg / mL, this indicates that the antioxidant activity of quercetin is a strong antioxidant. Meanwhile, turmeric (*Curcuma longa* L.) has a weak antioxidant activity with an IC50 value of 155.780 µg / mL and curcuma (*Curcuma xanthorrhiza* Roxb.) Has a strong antioxidant activity with an IC value of 26.386 µg / mL, this indicates that the ethanol extract of turmeric (*Curcuma longa* L.) has different antioxidant activity with the ethanol extract of curcuma (*Curcuma xanthorrhiza* Roxb.) [14]. The results of this study indicate that the level of antioxidant activity of the ethanol extract of turmeric (*Curcuma longa* L.) and ethanol extract of Curcuma (*Curcuma xanthorrhiza* Roxb.) from Maros Regency of South Sulawesi has different levels of antioxidant activity with the ethanol extract of turmeric (*Curcuma longa* L.) and ethanol extract. Curcuma (*Curcuma xanthorrhiza* Roxb.) originating from the Surakarta area of Central Java which is the result of research from [6], although it comes from the same species. This is influenced by plant age, cultivation method, environmental conditions where it is grown (geographic origin), harvest time, and post-harvest and storage processes [1] [15].

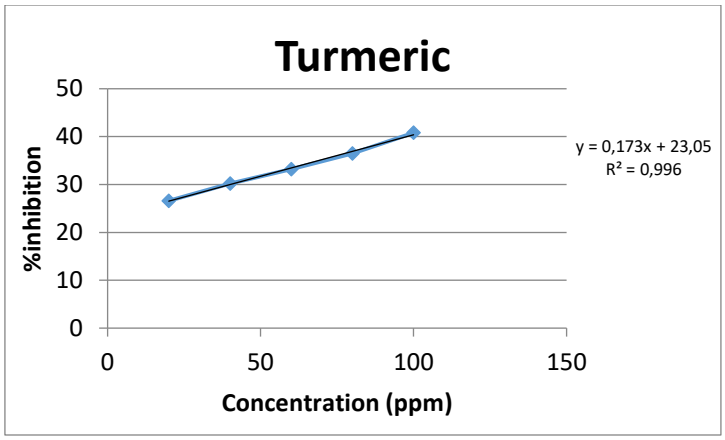

FIGURE 4. Relationship between the concentration of the ethanol extract of Curcuma (*Curcuma xanthorrhiza* Roxb.) and the percentage of DPPH inhibition

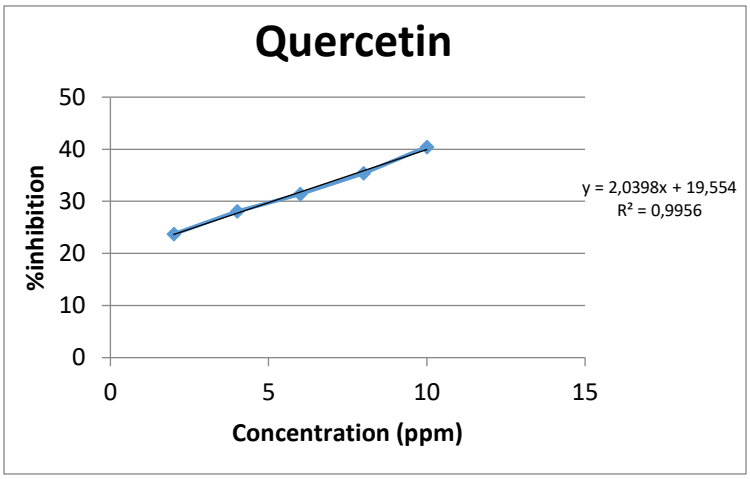

FIGURE 5. Relationship between the comparable concentration of quercetin and the percentage of DPPH inhibition

CONCLUSION

The ethanol extract of turmeric (*Curcuma longa* L.) and the ethanol extract of Curcuma (*Curcuma xanthorrhiza* Roxb.) produces varying values of TPC, TFC, and IC50. Samples containing phenolic and flavonoid as well as the highest antioxidant activity compared to other samples. Judging from the resulting loading plot and scatter plot, there is a strong negative correlation between IC50 DPPH and phenolic content, which indicates that the higher the phenolic content, the higher the antioxidant activity; moderate negative correlation between IC50 DPPH and flavonoid content, which indicates that the higher the flavonoid content, the higher the antioxidant activity produced; and a strong positive correlation between phenolic content and flavonoid content. The ethanol extract of turmeric (*Curcuma longa* L.) has weak antioxidant activity with an IC value of 155.780 µg / mL and the ethanol extract of Curcuma (*Curcuma xanthorrhiza* Roxb.) has a strong antioxidant activity with an IC value of 26.386 µg /mL.

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